Journal of the Saudi Society of Agricultural Sciences (2015) xxx, xxx–xxx

King Saud University

Journal of the Saudi Society of Agricultural Sciences

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FULL LENGTH ARTICLE

# Radical scavenging potential and DNA damage protection of wild edible mushrooms of Kashmir Himalaya

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Received 6 September 2015; revised 16 September 2015; accepted 25 October 2015

**KEYWORDS**

Antioxidant activity;
Mushroom;
DNA;
Radicals;
Ethanol

**Abstract** The edible mushrooms *Verpa bohemica* and *Morchella esculenta* are locally used for dietary and antioxidant in tribal areas of Kashmir Himalaya. In the present study, sequences of solvents on the basis of their polarity were used for the extraction from selected mushrooms. The comprehensive antioxidant activity of all edible mushroom extracts was evaluated by seven different methods. *V. bohemica* exhibited significant inhibitory activity of radicals among all the mushrooms while *Morchella* extracts protected the DNA damage from OH· radicals. This study provides us the substantiation for the use of these mushrooms as antioxidants besides being already eaten as food.


## 1. Introduction

Free radicals are considered to be the important candidates for the development of various diseases in humans. The dearth in endogenous antioxidant defense may result in oxidative stress, associated with various health problems, including heart diseases, neurological disorders, diabetes, arthritis and leukemia (Smolskait et al., 2015). However, dietary antioxidants particularly those obtained from plant biosystems are believed to assist in maintaining good health, as well as in preventing various diseases (Augustyniak et al., 2010). Mushrooms have been widely used as a human food for centuries and have been acceptable for texture and flavor. However, the knowledge of mushrooms as being an important source of biological active substances with medicinal value has only recently emerged (Nowacka et al., 2014). A number of mushroom species has been reported during last decade to possess significant antioxidant activity (Kalogeropoulos et al., 2013). Mushroom and its

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Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jssas.2015.10.005

metabolites have multibeneficial effects for human welfare for the treatment of various diseases and related health problems (Kalogeropoulos et al., 2013). Mushroom research has focused on discovery of compounds that can modulate positively or negatively the biological response of immune cells and it attracts an enormous international attention as a valuable herb due to the wide variety of its biological activities, such as antimicrobial, anticancer, antidiabetes, and hepatoprotective activities (Lindequist et al., 2005; Kalogeropoulos et al., 2013). In this backdrop, two edible mushroom species *Verpa* and *Morchella esculenta* from the same family Morchellaceae and division ascomycota were selected on the basis of their common usage in mountainous and hilly areas of Kashmir Himalaya (India). There are some reports for the use of mushrooms in the region and some preliminary work has also been carried out regarding the nutritional and antioxidant potential of other mushrooms whose citations are given below, but our study is first of its kind to evaluate the comparative antioxidant activity of the *V. bohemica* and *M. esculenta* (Wani et al., 2010; Boda et al., 2012). *V. bohemica* is a medium-sized (3-in. tall), tannish mushroom with a wrinkled, bell-shaped cap which is attached only at the top, forming a skirt over the stem. It is considered among the better as for its edibility however if eaten in excess causes gastrointestinal upsets and loss of muscular coordination. *M. esculenta* (commonly known as common morel, morel, yellow morel, true morel, morel mushroom, and sponge morel) is one of the most readily recognized and highly prized of all the edible mushrooms. *M. esculenta* fruit bodies have several medicinal properties; including anti-tumor effects, immunoregulatory properties, (Duncan et al., 2002), fatigue resistance, and antiviral effect (Nitha and Janardhanan, 2008; Rotzoll et al., 2005). Polysaccharides from *M. esculenta* mycelia have been reported for antioxidant activity (Mau et al., 2004; Elmastas et al., 2006; Gursoy et al., 2009). Morels are also used in traditional Chinese medicine to treat indigestion, excessive phlegm, and shortness of breath (Ying et al., 1987). In this context, the present study was carried out to evaluate the comprehensive radical scavenging potential of two edible mushrooms found in north western Himalaya. To our knowledge, this work is first in evaluating the selected mushroom species for antioxidant activities in the Himalayan area.

## 2. Materials and methods

### 2.1. Chemicals required

Solvents and chemicals were procured from standard companies such as Merck and *Hi-media* Mumbai Pvt. Ltd.

### 2.2. Collection and identification of mushroom species

*V. bohemica* and *M. esculenta* species are present in the range of 1800–4000 m in North Western Himalaya and in this study the mushrooms were collected manually in bulk from Aparhwat range (Gulmarg area 3748 m asl), Kashmir Himalaya, J&K. *V. bohemica* was collected in the ending week of April while *M. esculenta* was collected in the month of May and first week of June in the period from April–June, 2014. All the mushrooms were collected at fully mature stage with complete fruit body formation. The species were identified under accession No. 1848 by Kashmir University Herbarium (KASH), Centre of Plant Taxonomy, Department of Botany at University of Kashmir, Srinagar.

### 2.3. Preparation of mushroom extracts

All the mushroom species were shade dried at the temperature of 30 °C in AC fitted room with dehumidifiers so as to protect them from the disease due to their higher water content. The dried powder (100 g) of each mushroom species was subjected to rigorous extraction via soxhlet apparatus. Thereafter, the material was pre-extracted with petroleum ether to remove oily substances. The resulting petroleum ether extract contained large amounts of secondary metabolites. The pre-extracted extracts were then refluxed with ethyl acetate, butanol, ethanol, methanol followed by aqueous. Extracts were concentrated using rotary evaporator, which were later completely dried, weighed and kept for further usage in sterilized sealed vials at 4 °C. With the aim of optimization in the complete extraction of samples, effects of different extraction solvents were premeditated via ultrasound-assisted extraction as a fast and proficient extraction tool. Five solvents with diverse polarity such as ethyl acetate, ethanol, butanol, aqueous and methanol had been selected based on prior studies (Nowacka et al., 2014).

### 2.4. Antioxidant activity

#### 2.4.1. DPPH assay

Quantitative measurement of radical scavenging properties of extracts was carried out according to the method (Parray et al., 2015b). 0.1 mM solution of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in methanol was prepared and 1 ml of this solution was added to 3 ml of extract (200–800 µg/ml). Ascorbic acid and BHT were used as reference antioxidants. Discoloration of reaction mixture was measured at 517 nm after incubation for 30 min.

#### 2.4.2. Superoxide anion radical scavenging activity

Measurement of superoxide anion scavenging activity of the all extracts was carried out based on the method (Parray et al., 2015b) with slight modification. 100 µl riboflavin solution (20 µg), 200 µl EDTA solution (12 mM), 200 µl methanol and 100 µl nitro-blue tetrazolium (NBT) solution (0.1 mg) were mixed in test tube and reaction mixture was diluted up to 3 ml with phosphate buffer (50 mM). The absorbance of solution was measured at 590 nm using phosphate buffer as blank after illumination for 5 min. Different concentrations (50 µl), i.e. 100–800 µg/ml of extracts were taken and diluted up to 150 µl with methanol. To each of these, add 100 µl riboflavin, 200 µl EDTA, and 200 µl methanol and 100 µl NBT was mixed in test tube and further diluted up to 3 ml with phosphate buffer. Absorbance was measured after illumination for 5 min at 590 nm. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity.

#### 2.4.3. Hydroxyl scavenging activity-deoxyribose assay

The colorimetric deoxyribose (TBARS) method (Parray et al., 2015b) was applied for determining the hydroxyl radical scavenging activity of extracts. The reaction mixture for the

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deoxyribose assay contained in a final volume of 1 ml of the following reagents: 200 µl $KH_2PO_4$-KOH (100 mM), 200 µl deoxyribose (15 mM), 200 µl Ferric chloride (500 µM), 100 µl EDTA (1 mM), 100 µl ascorbic acid (1 mM), 100 µl Hydrogen peroxide (10 mM), and 100 µl of plant sample (100–300 µg/ml). Reaction mixtures were incubated at 37 °C for 1 h. At the end of the incubation period, 1 ml of 1% (w/v) thiobarbituric acid (TBA) was added to each mixture followed by the addition of 1 ml of 2.8% (w/v) trichloroacetic acid (TCA). The solutions were heated on a water-bath at 80 °C for 20 min to develop pink colored malondialdehyde-thiobarbituric acid (MDA-TBA) adduct and the absorbance of the resulting solution (total volume = 3 ml) was measured at 532 nm.

#### 2.4.4. Ferric Thiocyanate (FTC) (Parray et al., 2015b)

*In this method* 2 ml of extract (100–300 µg/ml) was mixed with 2.88 ml of linoleic acid (2.51%, v/v in 4 ml of 99.9% ethanol), 0.05 M phosphate buffer (pH 7.0, 8 ml) and distilled water (3.9 ml). The whole reaction mixture was followed by 0.1 ml of 30% ammonium thiocyanate. Immediately, after 3 min, 0.1 ml of 3.5% v/v HCl was added to the reaction mixture, the absorbance at 500 nm of the resulting solution was measured and it was recorded again after 24 h, until the day when the absorbance of the control reached the maximum value. Ascorbic acid and BHT were used as reference antioxidants.

#### 2.4.5. Thiobarbituric acid assay

Thiobarbituric acid was added to the reaction mixture which interacts with malondialdehyde and TBARS produced was measured spectrophotometrically (Parray et al., 2015b). To 2 ml of reaction mixtures of ferric thiocyanate assay, 2 ml of TCA (20%) and 2 ml of TBA (0.67%) were added and kept in boiling water for 10 min and were later on cooled under tap water. The reaction mixture was centrifuged at 3000 rpm for 20 min and the supernatant was read at 500 nm. Ascorbic acid and BHT were used as reference antioxidants.

#### 2.4.6. Lipid peroxidation method

A modified thiobarbituric acid reactive species (TBARS) assay (Parray et al., 2015b) was used to measure the lipid peroxide formed using the egg yolk homogenate as lipid rich media. Malondialdehyde, a secondary end-product of oxidation of polyunsaturated fatty acids reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen. Egg homogenate (0.5 ml of 10% v/v) and 0.1 ml of plant extracts were added to a test tube and volume was made up to 1 ml with distilled water. The peroxidation was induced by adding 0.05 ml of 0.07 M $FeSO_4$. The reaction mixture was vortexed and then heated at 95 °C for 60 min. After cooling, 5 ml of butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the upper organic layer was measured at 532 nm. Ascorbic acid and BHT were used as reference antioxidants.

#### 2.4.7. Reducing power (RP) method

In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride measured at 700 nm (Oyaizu, 1986). 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) are added to 1.0 mL of sample dissolved in distilled water. The resulting mixture is incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of Trichloroacetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), and mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). Ascorbic acid and BHT were used as reference antioxidants.

### 2.5. Calculations

The capacity to scavenge the radicals was calculated using the following equation:

$$\% \text{ inhibition} = A_c - A_s/A_c \times 100$$

where '$A_c$' is the absorbance of the controlled reaction (reaction mixture without any antioxidant substance) and '$A_s$' is absorbance of reaction mixture with reference substance or extract. The whole experiments were repeated thrice.

### 2.6. DNA damage assay

In this method, hydroxyl radicals generated by Fenton's reaction were used to induce oxidative damage to DNA. The reaction mixture (15 µL) contained 25 mg of calf thymus DNA (purchased from Sigma Aldrich, it is prepared from calf thymus tissue and It is used as a standard to determine DNA content in gels and for DNA quantification or other assays.) in 20.0 mM phosphate buffer saline (pH 7.4) and 500 µg of test compounds were added and incubated with DNA for 15 min at room temperature. The oxidation was induced by treating DNA with 1 µl $H_2O_2$ (30 mM), 1 µl (20 mM) ferric nitrate and 1 µl (100 mM) ascorbic acid and incubated them for 1 h at 37 °C. The reaction was terminated by the addition of loading dye (40% sucrose and 0.25% bromophenol blue) and the mixture was subjected to gel electrophoresis in 0.7% agarose/TAE buffer run at 100 V. DNA was visualized by Gel Doc system (Parray et al., 2015b).

### 2.7. Statistical analysis

Data were subjected to analysis of variance using SPSS software version 17.0 (SAS Institute Inc., Cary, NC, USA). The inhibitory activity of mushroom extracts in different methods was considered significant according to the magnitude of the $F$ value ($< 0.005$).

#### 2.7.1. PCA

The Principal Component Analysis (PCA) using SPSS 17.0 software was applied and test was done with respect to higher concentration and antioxidant method.

## 3. Results and discussion

In the present study, extracts from the edible mushrooms were evaluated for free radical scavenging activity. The mushrooms were macerated with five solvents, i.e. ethyl acetate, butanol, ethanol, methanol and aqueous in a sequential manner on the basis of polarity (Parray et al., 2010, 2011). A single analytical method may not reveal the total antioxidant capacity of a group of compounds, since different antioxidants may

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function by different mechanisms and also the bioactive substances present in mushroom extracts may be different. In this regard, seven different antioxidant methods were simultaneously used for assessing the scavenging potential of mushroom species, (Parray et al., 2015a,b).

The results found that FTC assay among all the methods was found effective for evaluation of the inhibitory activity of mushroom extracts. The ethanol extract followed by methanol and butanol of all the species was also found effective in scavenging of radicals. The antioxidant activity of all the extracts increased with the increment in tested concentration and in continuum with our study, and Lee et al. (2007) also reported increase in antioxidant activities of *Pleurotus citrinopileatus* with increased concentration. The ethanol extract of *V. bohemica* at 800 μg/mg showed maximum scavenging of radicals (93%) in FTC assay followed by 89% in TBA assay, 85% in reducing assay and 83% in lipid peroxidation method. The methanol extract of *V. bohemica* showed 92% free radical scavenging of DPPH and peroxy radicals at 800 μg/mg. Meanwhile, the butanol extract was also reported to scavenge about 90% of superoxide radicals and in other methods it showed moderate to mild activity. The ethyl acetate extract exhibited 88% scavenging of DPPH radicals followed by 74% of scavenging of thiocyanate radicals at higher concentration. The DPPH radical scavenging assay is a widely accepted model to assess free radical-scavenging activities (Reis et al., 2011). It was observed from the results that ascorbic acid and BHT used as positive control showed good activity but in some cases the activity of mushroom extracts was significant ($P < 0.05$) (Table 1).

The methanol extract (800 μg/mg) of *V. bohemica* showed 92% free radical scavenging of DPPH and peroxy radicals followed by 85% inhibition of radicals in FTC assay. The methanol extract of the plant at higher conc. was found to scavenge about 92% peroxy radicals and 91% superoxide radicals. The ethyl acetate extract also showed good scavenging activity (89%) of DPPH radicals followed by 82% scavenging of superoxide radicals. The butanol and aqueous extracts showed very low inhibition (Table 1). The activity of *Morchella esculenta* was found lower among all the other mushroom extracts. As reported above, the scavenging potential of ethanol extract of *M. esculenta* (800 μg/mg) also exhibited significant scavenging of radicals (92%) in reducing assay followed by 75% inhibition of radicals in FTC assay. The methanol extract was also observed to exhibit 78% scavenging in reducing assay while butanol extract exhibited 79% quenching of hydroxyl radicals. All other extracts exhibited very low to moderate inhibition of radicals in various antioxidant methods (Table 2). The aqueous extract from all the species exhibited the least inhibition of radicals in all the methods. The radical scavenging activity of the tested mushroom species is in accordance with the findings of plant scientists working on wild mushrooms in around the world (Boda et al., 2012; Kumar et al., 2013; Obodai et al., 2014). Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity of the plants (Kumar et al., 2013). The results pertaining to radical scavenging potential clearly emphasize the use of these edible mushrooms by large folk of the Himalayan area.

In the present study, $IC_{50}$ was also determined for all the antioxidant methods. The $IC_{50}$ values varied with the extract

**Table 1** Antioxidant activity of different solvent extracts of *Verpa bohemica*.

| Method | Conc (μg/mg) | % Scavenging of radicals | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Ethyl acetate | Butanol | Ethanol | Methanol | Aqueous | BHT | Ascorbic acid |
| DPPH assay | 200 | 63 ± 4.56$^{e}$ | 28 ± 1.5$^{a}$ | NA | 45 ± 5.43$^{b}$ | NA | 60 ± 2.26$^{c}$ | 67 ± 3.66$^{c}$ |
| | 400 | 72 ± 2.16$^{ef}$ | 35 ± 2.56$^{a}$ | NA | 65 ± 2.23$^{d}$ | NA | 73 ± 3.16$^{d}$ | 78 ± 2.65$^{de}$ |
| | 800 | 88 ± 3.5$^{g}$ | 43 ± 3.12$^{b}$ | 25 ± 2.6$^{a}$ | 92 ± 3.56$^{f}$ | 32 ± 2.54$^{a}$ | 89 ± 3.36$^{e}$ | 87 ± 1.32$^{e}$ |
| SOD assay | 100 | NA | 41 ± 3.33$^{b}$ | 23 ± 5.16$^{a}$ | 43 ± 3.24$^{b}$ | 41 ± 3.65$^{b}$ | 58 ± 4.56$^{b}$ | 60 ± 2.14$^{c}$ |
| | 300 | NA | 65 ± 5.09$^{d}$ | 36 ± 1.56$^{ab}$ | 55 ± 3.32$^{c}$ | 57 ± 1.34$^{c}$ | 68 ± 2.6$^{c}$ | 74 ± 2.23$^{d}$ |
| | 500 | 22 ± 3.2$^{a}$ | 90 ± 2.51$^{f}$ | 48 ± 1.0$^{bc}$ | 66 ± 1.14$^{d}$ | 68 ± 2.22$^{d}$ | 82 ± 3.26$^{e}$ | 80 ± 1.17$^{e}$ |
| Lipid peroxidation assay | 200 | 54 ± 1.36$^{d}$ | 57 ± 1.56$^{a}$ | 40 ± 3.41$^{b}$ | 32 ± 2.34$^{a}$ | 32 ± 1.56$^{a}$ | 50 ± 3.36$^{b}$ | 39 ± 5.83$^{a}$ |
| | 400 | 66 ± 5.46$^{e}$ | 60 ± 2.3$^{d}$ | 69 ± 3.21$^{de}$ | 53 ± 3.56$^{c}$ | 39 ± 3.09$^{ab}$ | 63 ± 3.32$^{c}$ | 55 ± 2.43$^{b}$ |
| | 800 | 74 ± 6.2$^{f}$ | 65 ± 2.56$^{d}$ | 83 ± 4.33$^{f}$ | 92 ± 3.43$^{f}$ | 48 ± 2.12$^{bc}$ | 72 ± 3.44$^{d}$ | 62 ± 3.19$^{c}$ |
| Reducing assay** | 100 | 23 ± 3.08$^{a}$ | 36 ± 4.08$^{a}$ | 45 ± 5.31$^{b}$ | 34 ± 1.33$^{a}$ | 40 ± 3.34$^{a}$ | 59 ± 3.53$^{bc}$ | 40 ± 4.87$^{a}$ |
| | 300 | 34 ± 3.12$^{b}$ | 61 ± 3.22$^{cd}$ | 63 ± 3.43$^{d}$ | 49 ± 3.21$^{a}$ | 58 ± 2.13$^{c}$ | 68 ± 3.18$^{c}$ | 58 ± 2.33$^{b}$ |
| | 500 | 39 ± 3.02$^{bc}$ | 75 ± 3.16$^{e}$ | 85 ± 2.93$^{f}$ | 55 ± 2.12$^{c}$ | 70 ± 3.56$^{e}$ | 80 ± 3.5$^{e}$ | 75 ± 3.67$^{d}$ |
| FTC assay** | 200 | 45 ± 1.56$^{c}$ | 46 ± 2.72$^{b}$ | 64 ± 3.18$^{d}$ | 51 ± 3.43$^{c}$ | NA | 56 ± 3.34$^{b}$ | 68 ± 3.43$^{c}$ |
| | 400 | 65 ± 2.56$^{e}$ | 58 ± 3.83$^{c}$ | 79 ± 2.29$^{e}$ | 62 ± 3.32$^{d}$ | 39 ± 1.67$^{ab}$ | 67 ± 3.32$^{c}$ | 79 ± 4.56$^{e}$ |
| | 800 | 74 ± 2.45$^{f}$ | 64 ± 3.44$^{d}$ | 93 ± 1.06$^{g}$ | 79 ± 4.19$^{e}$ | 48 ± 3.33$^{bc}$ | 83 ± 3.19$^{e}$ | 89 ± 3.56$^{e}$ |
| TBA assay | 200 | 38 ± 1.32$^{b}$ | 43 ± 2.5$^{b}$ | 52 ± 1.36$^{c}$ | 45 ± 3.43$^{a}$ | NA | 44 ± 3.56$^{a}$ | 53 ± 3.43$^{b}$ |
| | 400 | 53 ± 2.33$^{d}$ | 49 ± 6.07$^{bc}$ | 60 ± 2.29$^{d}$ | 53 ± 3.21$^{c}$ | NA | 50 ± 3.09$^{b}$ | 67 ± 3.56$^{c}$ |
| | 800 | 68 ± 4.10$^{e}$ | 55 ± 5.14$^{c}$ | 89 ± 3.33$^{fg}$ | 64 ± 2.17$^{d}$ | 33 ± 3.54$^{a}$ | 65 ± 2.33$^{c}$ | 74 ± 2.14$^{a}$ |
| Hydroxyl scavenging assay | 200 | NA | 31 ± 3.23$^{a}$ | 27 ± 4.43$^{a}$ | 30 ± 3.22$^{a}$ | 50 ± 3.90$^{c}$ | 60 ± 3.43$^{c}$ | 65 ± 2.23$^{c}$ |
| | 400 | 27 ± 2.5$^{a}$ | 38 ± 2.12$^{ab}$ | 39 ± 5.31$^{b}$ | 39 ± 1.89$^{ab}$ | 59 ± 3.32$^{cd}$ | 67 ± 1.56$^{c}$ | 70 ± 3.65$^{d}$ |
| | 600 | 35 ± 3.5$^{b}$ | 43 ± 1.43$^{a}$ | 56 ± 4.12$^{c}$ | 69 ± 2.12$^{de}$ | 61 ± 3.43$^{d}$ | 81 ± 2.56$^{e}$ | 89 ± 2.44$^{e}$ |

Values are represented as mean ± SD ($n = 3$), and data were analyzed by ANOVA using Duncan's multiple range test (SPSS 17.0); the values with different superscript along the columns are statically significant at $P < 0.005$.

** Values represent third day reading; NA = No activity.

**Table 2** Antioxidant activity of different solvent extracts of *Morchella esculenta*.

| Method | % Scavenging of radicals | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Conc. (μg/mg) | Ethyl acetate | Butanol | Ethanol | Methanol | Aqueous | BHT | Ascorbic acid |
| DPPH assay | 200 | NA | NA | 34 ± 1.44$^{a}$ | NA | NA | 60 ± 2.26$^{c}$ | 67 ± 3.66$^{c}$ |
| | 400 | NA | NA | 43 ± 2.26$^{b}$ | NA | NA | 73 ± 3.16$^{d}$ | 78 ± 2.65$^{de}$ |
| | 800 | 23 ± 1.23$^{a}$ | 28 ± 2.68$^{a}$ | 65 ± 3.54$^{d}$ | 29 ± 3.12$^{a}$ | 21 ± 4.16$^{a}$ | 89 ± 3.36$^{e}$ | 87 ± 1.32$^{e}$ |
| SOD assay | 100 | 40 ± 2.87$^{c}$ | 35 ± 2.32$^{b}$ | 29 ± 2.28$^{a}$ | 33 ± 3.16$^{a}$ | NA | 58 ± 4.56$^{b}$ | 60 ± 2.14$^{c}$ |
| | 300 | 54 ± 1.65$^{d}$ | 43 ± 3.14$^{c}$ | 43 ± 3.49$^{b}$ | 55 ± 3.94$^{c}$ | NA | 68 ± 2.6$^{c}$ | 74 ± 2.23$^{d}$ |
| | 500 | 67 ± 3.98$^{e}$ | 58 ± 1.18$^{d}$ | 60 ± 3.21$^{c}$ | 63 ± 3.03$^{d}$ | 35 ± 3.36$^{b}$ | 82 ± 3.26$^{e}$ | 80 ± 1.17$^{e}$ |
| Lipid peroxidation assay | 200 | 34 ± 1.36$^{b}$ | 29 ± 3.04$^{a}$ | 40 ± 3.85$^{b}$ | 38 ± 2.09$^{b}$ | NA | 50 ± 3.36$^{b}$ | 39 ± 5.83$^{a}$ |
| | 400 | 38 ± 3.43$^{b}$ | 35 ± 1.54$^{b}$ | 46 ± 2.98$^{bc}$ | 45 ± 3.10$^{b}$ | 27 ± 3.87$^{a}$ | 63 ± 3.32$^{c}$ | 55 ± 2.43$^{b}$ |
| | 800 | 49 ± 1.29$^{c}$ | 41 ± 3.32$^{c}$ | 51 ± 2.21$^{c}$ | 51 ± 4.19$^{c}$ | 31 ± 1.56$^{ab}$ | 72 ± 3.44$^{d}$ | 62 ± 3.19$^{c}$ |
| Reducing assay** | 100 | 34 ± 3.09$^{b}$ | 40 ± 3.69$^{c}$ | 44 ± 3.69$^{b}$ | 44 ± 3.10$^{b}$ | 61 ± 1.06$^{e}$ | 68 ± 3.53$^{c}$ | 40 ± 4.87$^{a}$ |
| | 300 | 48 ± 1.43$^{c}$ | 55 ± 1.21$^{d}$ | 65 ± 4.33$^{d}$ | 64 ± 2.06$^{d}$ | 69 ± 2.14$^{ef}$ | 59 ± 3.18$^{bc}$ | 58 ± 2.33$^{b}$ |
| | 500 | 69 ± 3.87$^{e}$ | 70 ± 3.72$^{f}$ | 92 ± 1.21$^{f}$ | 78 ± 2.98$^{a}$ | 71 ± 3.16$^{f}$ | 80 ± 3.5$^{e}$ | 75 ± 3.67$^{d}$ |
| FTC assay** | 200 | NA | 39 ± 2.43$^{bc}$ | 55 ± 3.09$^{c}$ | 48 ± 3.90$^{bc}$ | NA | 56 ± 3.34$^{b}$ | 68 ± 3.43$^{c}$ |
| | 400 | 46 ± 2.8$^{c}$ | 48 ± 4.22$^{c}$ | 67 ± 2.38$^{de}$ | 57 ± 3.19$^{c}$ | NA | 67 ± 3.32$^{c}$ | 79 ± 4.56$^{e}$ |
| | 800 | 58 ± 3.34$^{d}$ | 67 ± 3.04$^{e}$ | 75 ± 3.90$^{e}$ | 66 ± 2.17$^{d}$ | 59 ± 3.29$^{de}$ | 83 ± 3.19$^{e}$ | 89 ± 3.56$^{e}$ |
| TBA assay | 200 | NA | NA | 41 ± 4.08$^{b}$ | 34 ± 1.19$^{a}$ | NA | 44 ± 3.56$^{a}$ | 53 ± 3.43$^{b}$ |
| | 400 | 32 ± 4.3$^{b}$ | 32 ± 3.98$^{b}$ | 48 ± 3.54$^{c}$ | 49 ± 2.14$^{c}$ | NA | 50 ± 3.09$^{b}$ | 67 ± 3.56$^{c}$ |
| | 800 | 49 ± 3.15$^{c}$ | 54 ± 4.68$^{d}$ | 59 ± 3.59$^{d}$ | 60 ± 1.43$^{d}$ | 36 ± 2.08$^{b}$ | 65 ± 2.33$^{c}$ | 74 ± 2.14$^{a}$ |
| Hydroxyl scavenging assay | 200 | NA | 27 ± 3.28$^{a}$ | 40 ± 3.28$^{b}$ | 43 ± 1.54$^{b}$ | NA | 60 ± 3.43$^{c}$ | 65 ± 2.23$^{c}$ |
| | 400 | 27 ± 3.28$^{a}$ | 38 ± 4.49$^{b}$ | 56 ± 3.22$^{d}$ | 69 ± 1.93$^{de}$ | 36 ± 1.63$^{b}$ | 67 ± 1.56$^{c}$ | 70 ± 3.65$^{d}$ |
| | 600 | 72 ± 3.74$^{e}$ | 79 ± 3.61$^{f}$ | 70 ± 3.21$^{e}$ | 76 ± 3.36$^{e}$ | 42 ± 3.36$^{c}$ | 81 ± 2$^{e}$.56 | 89 ± 2.44$^{e}$ |

Values are represented as mean ± SD ($n$ = 3), and data were analyzed by ANOVA using Duncan's multiple range test (SPSS 17.0); the values with different superscript along the columns are statically significant at $P < 0.005$.
** Values represent third day reading; NA = No activity.

**Table 3** $IC_{50}$ (μg/mg) values of solvent extracts of *V. bohemica and M. esculenta*.

| | $IC_{50}$ (μg/mg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ethyl acetate | Butanol | Ethanol | Methanol | Aqueous | BHT | Ab |
| *Verpa bohemica* | | | | | | | |
| DPPH assay | < 200 | 1083.33 ± 7.87$^{j}$ | 334.09 ± 3.98$^{d}$ | 243.421 ± 5.98$^{bc}$ | 1157.8 ± 8.6$^{m}$ | < 200 | < 200 |
| SOD assay | 1075 ± 5.6$^{m}$ | 175.57 ± 5.34$^{a}$ | 525.69 ± 2.76$^{g}$ | 225.6 ± 3.56$^{b}$ | 222.6 ± 5.32$^{b}$ | < 100 | < 100 |
| Lipid Peroxidation assay | < 200 | < 200 | 257.57 ± 1.54$^{c}$ | 378.78 ± 5.12$^{g}$ | 865.38 ± 6.34$^{i}$ | 200$^{a}$ | 583.4 ± 4.3$^{d}$ |
| Reducing assay | 750 ± 4.16$^{j}$ | 225.97 ± 5.23$^{b}$ | 156.7 ± 5.13$^{a}$ | 379.8 ± 5.56$^{g}$ | 220 ± 5.6$^{b}$ | < 100 | 98.7 ± 5.87$^{a}$ |
| FTC assay | 215.9 ± 9.43$^{b}$ | 259.25 ± 5.98$^{c}$ | < 200 | 163.04 ± 3.45$^{a}$ | 812.2 ± 4.63$^{h}$ | < 200 | < 200 |
| TBA assay | 393.2 ± 4.76$^{g}$ | 526.3 ± 5.05$^{f}$ | < 200 | 338.7 ± 4.23$^{f}$ | 1146.5 ± 5.78$^{m}$ | 400$^{b}$ | < 200 |
| Hydroxyl scavenging assay | 739.42 ± 3.09$^{j}$ | 822.33 ± 4.6$^{i}$ | 533.4 ± 3.43$^{g}$ | 443.2 ± 5.6$^{h}$ | 200 ± 7.12$^{a}$ | < 200 | < 200 |
| *Morchella esculenta* | | | | | | | |
| DPPH assay | 1500 ± 12.6$^{a}$ | 1280 ± 7.6$^{l}$ | 519.23 ± 12.5$^{fg}$ | 1264.7 ± 5.6$^{m}$ | 1635.13 ± 5.33$^{a}$ | < 200 | < 200 |
| SOD assay | 247.61 ± 5.6$^{c}$ | 384.56 ± 5.6$^{d}$ | 379.87 ± 09.09$^{e}$ | 295.33 ± 5 ± 5.76$^{e}$ | 742.06 ± 5.09$^{f}$ | < 100 | < 100 |
| Lipid Peroxidation assay | 860 ± 5.12$^{l}$ | 1263.15 ± 2.6$^{l}$ | 735.294 ± 7.6$^{h}$ | 750 ± 5.48$^{k}$ | 1155.5 ± 5.45$^{m}$ | 200 ± 5.09$^{a}$ | 414.3 ± 5.19$^{c}$ |
| Reducing assay | 297.93 ± 4.12$^{d}$ | 233.33 ± 5.17$^{b}$ | 158.33 ± 3.98$^{a}$ | 158.82 ± 6.6$^{a}$ | < 100 | < 100 | 213.7 ± 5.6$^{b}$ |
| FTC assay | 643.67 ± 6.08$^{h}$ | 445.6 ± 3.45$^{e}$ | < 200 | 232.14 ± 6.32$^{b}$ | 757.14 ± 5.34$^{g}$ | < 200 | < 200 |
| TBA assay | 812.3 ± 3.23$^{k}$ | 717.64 ± 5.65$^{h}$ | 500 ± 5.76$^{f}$ | 524.39 ± 5.98$^{i}$ | 1062.5 ± 5.24$^{l}$ | 400 ± 3.54$^{b}$ | < 200 |
| Hydroxyl scavenging assay | 690.72 ± 2.17$^{i}$ | 641.15 ± 4.34$^{g}$ | 328.9 ± 5.3$^{d}$ | 248.04 ± 3.54$^{c}$ | 323.8 ± 5.98$^{c}$ | < 200 | < 200 |

Values are represented as mean ± SD ($n$ = 3), and data were analyzed by ANOVA using Duncan's multiple range test (SPSS 17.0); the values with different superscript along the columns are statically significant at $P < 0.005$. ND = Not detected within the range tested, Ab = Ascorbic acid.

as well as with the type of method employed. The ethanol extract of *V. bohemica* showed least value of 50% inhibition of radicals ($IC_{50}$ = 156.7 μg/mg) in reducing assay followed by methanol extract ($IC_{50}$ = 163.04 μg/mg) in FTC assay (Table 3). The ethanol extract of *M. esculenta* showed lowest value ($IC_{50}$ 158 μg/mg) in scavenging of radicals in reducing assay followed by $IC_{50}$ = 232.14 μg/mg by methanol extract in FTC assay (Table 3).

The mushroom extracts were found as an excellent radical scavengers and reducing agents. Antioxidant properties of edible wild mushrooms are of great interest in both academia and the food industry, since their possible use as natural additives

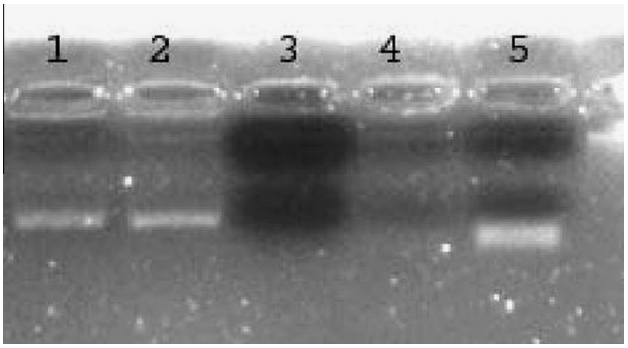


**Figure 1** Protective effect of DNA through scavenging of radicals by different extracts of *Morchella esculenta*. Lane 1: Native calf thymus (ct) DNA + Ethyl acetate extract (800 μg/mg) + reaction mixture. Lane 2: Native calf thymus (ct) DNA + Butanol extract (800 μg/mg) + reaction mixture. Lane 3: Native calf thymus (ct) DNA + Methanol extract (800 μg/mg) + reaction mixture. Lane 4: Native calf thymus (ct) DNA + reaction mixture. Lane 5: Native Calf thymus DNA.

(Wong and Chye, 2009). Owing to its excellent protective features exhibited in antioxidant activities, the extracts of edible wild mushrooms can emerge from a growing tendency to replace synthetic antioxidants. Edible wild sources may bring new natural products into the food industry with safer and better antioxidant activity that provides good protection against the oxidative damage, which occurs in both the body and our daily foods (Mau et al., 2004). In addition, these wild species have been consumed for decades by the locals without any reported side effects (Wong and Chye, 2009). Antioxidant properties of several specialty and edible mushrooms have been studied earlier (Mishra et al., 2013a; Obodai et al., 2014). There has been no report on extensive antioxidant activities of the studied mushrooms from Kashmir Himalaya. The high reducing power exhibited by the sample might be indicative of the hydrogen donating ability of the active species present in the extracts (Mishra et al., 2013b).

The *M. esculenta* extracts were also tested for their protective ability against DNA degradation by hydroxyl radicals. The reaction mixture (ferric nitrate, ascorbic acid and $H_2O_2$) completely induces DNA strand breaks in calf thymus DNA in our study. The *Morchella* extracts (ethyl acetate, butanol and methanol at 800 μg/mg) were able to scavenge the hydroxyl radicals generated by Fenton's reaction (lanes 1, 2 and 3) (Fig. 1) to protect the DNA degradation. Our results indicate that mushroom extracts showed strong DNA damage protection compared with that of natural antioxidant used. Hydroxyl radical is the most toxic radical known, as it can non-specifically oxidize all classes of biological macromolecules at virtually diffusion-limited rates (Ozyurek et al., 2014). Thus, hydroxyl radical scavenging activity is very important for evaluating the antioxidant activity of food substances so as to evaluate their nutraceutical value.

Free radical scavenging effect is a recognized phenomenon in inhibiting lipid oxidation, which otherwise can be deleterious to the cellular components and cellular function (Puttaraju et al., 2006). Natural antioxidant can be used to replace the synthetic antioxidant in the food industry such as BHT and ascorbic acid which may possess mutagenic activity (Skrinjar et al., 2007). In the present study the encouraging results about the radical scavenging activity and DNA protection potential, the consumption of wild edible mushrooms might be beneficial to protect human body against oxidative damage, which can be further developed into health related degenerative illnesses (Wong and Chye, 2009).

The PCA carried out signifies the particular method and the concentration of the specific extract among the edible mushrooms having effective radical scavenging capacity. All the methods except the DPPH assay were significant in evaluating the antioxidant potential of extracts (Fig. 2a and b).

In the present study we have observed that the ethanol fraction of both the mushrooms, i.e. *V. bohemica* and *M. esculenta* showed significant and encouraging inhibition of radicals. The ethanol fractions could be further subjected for UPLC-MS-MS for elucidation of different components in it.

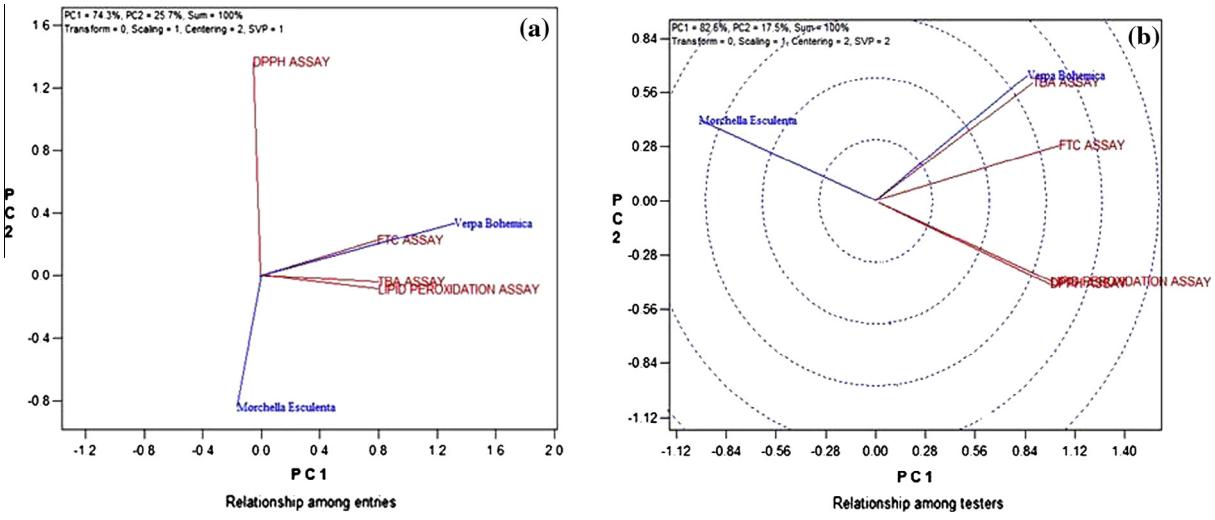


**Figure 2** (a) and (b): PCA of different constituents of edible mushrooms *Verpa bohemica* and *Morchella esculenta* with respect to higher concentration and method of determination.

## 4. Conclusions

The overall scavenging potential of mushrooms provides us the evidence for the use of these wild mushrooms as antioxidants besides being already eaten as food. They might be proposed as a dietary supplement or traditional drug for the prevention and/or treatment of conditions that occur due to oxidative damage or can protect DNA damage by hydroxyl radicals.

## Conflict of interest

The authors hereby declare that they have no conflict of interest.

## Author's contributions

All authors equally participated in designing experiments analysis and interpretation of data. All authors read and approved the final manuscript.

## Acknowledgments

This study was supported by DBT, GoI, New Delhi, and funded by women entrepreneurship project vide letter no: No. BT/PR6980/SPD/11/1417/2012 Dtd.26-09-2012 the assistance of which is highly acknowledged.

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Please cite this article in press as: Shameem, N. et al., Radical scavenging potential and DNA damage protection of wild edible mushrooms of Kashmir Himalaya. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.10.005